Resonance Raman spectroscopy of the oxygenated intermediates of human CYP19A1 implicates a Compound I intermediate in the final lyase step

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Supporting Information

EXPERIMENTAL

Materials

Androstenedione (AD) and 19-oxo-AD (19oxoAD) were purchased from Sigma-Aldrich and Steraloids, respectively, and were used as a 25 mM stock solution in methanol.

Protein expression, purification and incorporation into Nanodiscs

CYP19A1 was expressed, purified and assembled into MSP1D1-POPC Nanodiscs as previously described.[1,2]

Preparation of samples for rR measurements

70 µL of 250 µM ferric CYP19A1, 6.25 µM methylviologen (Sigma-Aldrich), 500 µM substrate, 30 % (v/v) glycerol (or glycerol-d3 where appropriate) in 0.1 M potassium phosphate, pH 7.4 was de-aerated under a continuous flow of Argon for 5 minutes in a small glass vial. The protein was reduced with a 2.5 fold molar excess of sodium dithionite (Riedel-de Haën AG, Germany) and transferred to 5 mm O.D. NMR tubes (WG-5 ECONOMY, Wilmad) under anaerobic conditions. After incubation at room temperature for 10 minutes the reduced sample was cooled down in a dry ice-ethanol bath to -13 °C for 1 minute. Oxy-ferrous complexes were formed by bubbling $^{16}O_2$ or $^{18}O_2$ gas for 3-5 seconds, followed by rapid cooling in a -60 °C dry ice-ethanol bath. The samples were then flash frozen and stored in liquid N_2 until measurement.

The rR measurements

The resonance Raman spectra of oxy complexes of ND:CYP19 were acquired using a Spex 1269 spectrometer equipped with a Spec-10 LN-cooled detector (Princeton Instruments, NJ). The data were measured with 413.1 nm excitation line from a Kr^+ laser (Coherent Innova Sabre Ion Laser). The rR spectra were collected using back scattering (180°) geometry with the laser beam being focused by a cylindrical lens to form a line image on the sample. The laser power was adjusted to 1 mW or less. All measurements were done at 77 K and total collection time was 3-4 hrs in the high frequency region and 4-5 hrs in the low frequency region. The slit width was set at 150 µm and the 1200 g/mm grating was used. The NMR tubes were positioned into a double-walled quartz low temperature cell filled with liquid nitrogen. The sample tubes were spun to avoid local heating. Spectra were calibrated with fenchone (Sigma-Aldrich, WI) and processed with Grams/32 AI software (Galactic Industries, Salem, NH).

RESULTS

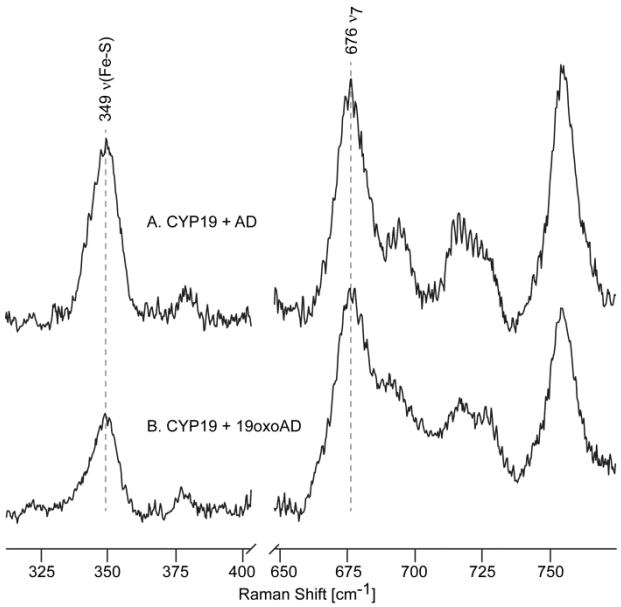

Figure S1. The low frequency spectra of ferric ND:CYP19 with AD (A) and 19-oxo-AD (B) substrates. Excitation line was 356.7 nm, the spectra were normalized to the ν_7 mode at 676 cm^{-1}. The lower intensity of the ν(Fe-S) mode in the spectrum of 19-oxo-AD reflects the lower high spin state population in this sample, as compared to the AD-bound sample.

REFERENCES

1. Gantt, S. L.; Denisov, I. G.; Grinkova, Y. V.; Sligar, S. G. *Biochem. Biophys. Res. Commun.* **2009**, *387*, 169.

2. Luthra, A.; Gregory, M.; Grinkova, Y. V.; Denisov, I. G.; Sligar, S. G. *Methods Mol. Biol.* **2013**, *987*, 115.